# DEPRESSION OF HEPATIC CYTOCHROME P-450-DEPENDENT MONOOXYGENASE SYSTEMS WITH ADMINISTERED INTERFERON INDUCING AGENTS

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SUMMARY: Cytochrome P-450-dependent monooxygenase activities and cytochrome P-450 levels were depressed in hepatic microsomes from rats treated with 12 interferon inducing agents of various types: small molecules (e.g. tilorone), an RNA virus (Mengo), a fungal mycophage (statolon), liver RNA, a synthetic double-stranded polynucleotide (poly rI · poly rC), a bacterial lipopolysaccharide (E. coli endotoxin) and an attenuated bacteria (B. pertussis vaccine). The results suggest that the depression of hepatic cytochrome P-450-dependent monooxygenase systems may be a general property of interferon inducing agents.

INTRODUCTION: Tilorone, 2,7-bis[2-(diethylamino)ethoxy]fluoren-9-one, is an antiviral agent which is thought to produce its effect by inducing interferon production (1,2). We reported recently that its administration to rats caused a marked depression of the hepatic cytochrome P-450-dependent monooxygenase systems responsible for the biotransformation of most drugs and other xenobiotics (3). Leeson et al. (4) reported similar results. These studies raised the question of whether the ability to depress these monooxygenase systems is a property peculiar to tilorone and related compounds, or whether it is a property of interferon inducing agents in general. In response to this question, twelve known interferon inducing agents representing a wide variety of structures and molecular weights, were tested for their effects on hepatic monooxygenase systems of the rat: an RNA virus (Mengo), a fungal mycophage (statolon), hepatic RNA, a synthetic double stranded polynucleotide (poly rI · poly rC), a bacterial lipopolysaccharide (E. coli endotoxin), an attenuated bacteria (B. pertussis vaccine), and six small molecules (quinacrine, N-N-dioctadecyl-n'n'-bis(2-hydroxyethyl)propanediamine, tilorone, and three derivatives of tilorone).

TABLE 1. Administration regimen of interferon inducing agents

| Agent | Dose (per kg body wt) | | Source | Reference[a] |
|---|---|---|---|---|
| Mengo virus | $5 \times 10^{-7}$ pfu | (iv) | b | 5 |
| Statolon | 50 mg | (ip) | Eli Lilly | 6 |
| Hepatic RNA (Type IV) | 5 mg | (ip) | Sigma | 7 |
| Poly rI · poly rC | 2.5 mg | (ip) | Sigma | 8 |
| *E. coli* endotoxin | 5 mg | (ip) | Sigma | 9 |
| *B. pertussis* vaccine | $2 \times 10^{11}$ cells | (ip) | Eli Lilly | 10 |
| Tilorone | 50 mg | (po) | Richardson-Merrill | 1 |
| RMI 11002[c] | 50 mg | (ip) | Richardson-Merrill | d |
| RMI 11567[e] | 50 mg | (ip) | Richardson-Merrill | d |
| RMI 11877[f] | 50 mg | (ip) | Richardson-Merrill | d |
| CP 20,901[g] | 50 mg | (ip) | Pfizer | 11 |
| Quinacrine | 50 mg | (ip) | Sigma | 12 |

[a]Selected reference to study showing interferon inducing activity of the agent.

[b]Supplied by Dr. P. G. W. Plagemann, Dept. of Microbiology, Univ. of Minnesota.

[c]3,6-bis(dimethylaminoacetyl)fluorene.

[d]Personal communication, Dr. G. J. Wright, Richardson-Merril, Inc., Cincinnati, Ohio.

[e]3,6-bis(dimethylaminoacetyl)dibenzofuran.

[f]3,6-bis(dimethylaminoacetyl)dibenzothiophene.

[g]N-N-dioctadecyl-n'n'-bis(2-hydroxyethyl)propanediamine

MATERIALS AND METHODS: The interferon inducing agents, their doses, routes of administration, sources, and references to their interferon inducing activities are given in Table 1. Male, Holtzman strain rats (180-200 g) were used. The agents were given daily for 3 days and microsomes were collected 24 hr after the last dose except in the case of Mengo virus, which was administered once and microsomes collected 24 or 48 hr later. Microsomes were prepared and assayed for their ethylmorphine N-demethylase and aniline p-hydroxylase activities and their cytochrome P-450, cytochrome $b_5$ and protein contents as described previously (3).

RESULTS: Table 2 summarizes the effects of the 12 interferon inducing agents (Table 1) on the ethylmorphine N-demethylase and aniline p-hydroxylase activi-

TABLE 2. Effects of interferon inducers on microsomal protein ethylmorphine N-demethylase, aniline p-hydroxylase, P-450 hemoprotein and cytochrome $b_5$

| INTERFERON INDUCER | MICROSOMAL PROTEIN (% of control) | ETHYLMORPHINE N-DEMETHYLASE (% of control) | ANILINE P-HYDROXYLASE (% of control) | P-450 HEMOPROTEIN (% of control) | CYTOCHROME $b_5$ (% of control) |
|---|---|---|---|---|---|
| Mengo virus 24 hr | 79.1 ± 9.7 | 73.3 ± 16.4 | 83.3 ± 9.8 | 77.1 ± 4.5[b] | 85.5 ± 6.1 |
| Mengo virus 48 hr | 46.0 ± 2.1[a] | 58.3 ± 1.4[a] | 82.6 ± 2.9[a] | 80.3 ± 8.1[b] | 107.1 ± 11.8 |
| Statolon | 84.5 ± 13.6 | 47.7 ± 6.9[a] | 60.8 ± 1.8[a] | 59.4 ± 1.8[a] | 81.1 ± 4.8[a] |
| Hepatic RNA (Type IV) | 91.0 ± 5.9 | 47.7 ± 6.4[a] | - - - - - - - | 69.5 ± 2.7[a] | 68.1 ± 5.6[a] |
| Poly rI · poly rC | 112.3 ± 18.9 | 53.5 ± 1.6[a] | 65.7 ± 5.7[a] | 64.8 ± 5.1[a] | 86.2 ± 2.0[a] |
| Poly rI | 124.7 ± 13.4 | 104.4 ± 6.4 | 94.0 ± 11.6 | 93.2 ± 4.7 | 97.9 ± 4.1 |
| Poly rC | 113.4 ± 7.8 | 88.1 ± 11.5 | 107.5 ± 10.8 | 94.6 ± 4.5 | 102.5 ± 2.6 |
| *E. coli* endotoxin | 105.5 ± 4.6 | 27.1 ± 4.8[a] | 69.6 ± 10.4[b] | 54.9 ± 5.3[a] | 79.2 ± 2.4[a] |
| *B. pertussis* vaccine | 120.0 ± 16.0 | 58.6 ± 11.9[a] | 68.5 ± 12.2[b] | 77.0 ± 6.0[a] | 79.8 ± 2.5[a] |
| Tilorone | 63.2 ± 5.1[a] | 50.5 ± 11.1[a] | 77.4 ± 7.1[a] | 64.5 ± 5.8[a] | 91.4 ± 8.5 |
| RMI 11002 | 90.1 ± 5.4 | 61.2 ± 9.7[a] | 61.6 ± 10.4[a] | 55.7 ± 5.9[a] | 78.9 ± 4.1 |
| RMI 11567 | 80.9 ± 5.4 | 72.1 ± 12.2 | 77.9 ± 10.9 | 72.0 ± 8.7[b] | 85.2 ± 5.5 |
| RMI 11877 | 66.1 ± 7.8[a] | 27.4 ± 9.1[a] | 47.1 ± 8.8[a] | 49.4 ± 7.6[a] | 80.6 ± 3.5 |
| CP 20,901 | 68.3 ± 6.9[a] | 42.0 ± 8.7[a] | 33.6 ± 7.4[a] | 44.8 ± 6.1[a] | - - - - - - - |
| Quinacrine | 93.2 ± 8.8 | 55.4 ± 1.5[a] | 53.9 ± 4.6[a] | 62.0 ± 7.0[a] | 89.7 ± 3.5 |

Values are the mean ± S.E. of the percentages of the individual control values for each agent; means ± S.E. of all of the control values were: microsomal protein, 11.28±0.68 mg/g of fresh liver; ethylmorphine N-demethylase, 306±22 nmol HCHO formed/mg of protein/ hr; aniline p-hydroxylase, 37.44±2.10 nmol p-OH-phenol formed/mg of protein/ hr; P-450 hemoprotein, 0.756±0.033 nmol/mg of protein; cytochrome $b_5$, 0.320±.016 nmol/mg of protein.

[a]Significantly different from corresponding control ($P < 0.05$).
[b]Significantly different from corresponding control ($P < 0.1$).

ties and the microsomal protein, P-450 hemoprotein and cytochrome $b_5$ contents of hepatic microsomes from the rat. Ethylmorphine N-demethylase and aniline p-hydroxylase activities and the cytochrome P-450 content of microsomes were depressed whenever an interferon inducing agent was administered, although in a few cases, the loss was significant only at the $P < 0.1$ level. Statistically significant losses of microsomal protein were seen only with tilorone and two of its analogues and with Mengo virus, which produced severe illness in the animals. Significant losses of cytochrome $b_5$ were observed with statolon, poly rI · poly rC, endotoxin and pertussis vaccine. Included in Table 2 are results obtained with the single stranded polynucleotides, poly rI and poly rC, neither of which is an interferon inducing agent (13). Neither compound depressed the microsomal activities and components under investigation.

It is not possible to determine the relative potencies of the agents listed in Table 2 as depressors of the monooxygenase systems because only a single dose of each was used and temporal aspects relative to administration and observance of effects were not considered. However, of the agents tested, E. coli endotoxin appeared to be the most potent and a dose response study was performed using mice. The i.p. administration of 40, 400, and 4000 µg of endotoxin/kg of mouse caused losses of microsomal ethylmorphine N-demethylase activity of 32, 47, and 63%, respectively. Corresponding losses of P-450 hemoprotein were 23, 48, and 45%. The lowest dose is equivalent to about 1 µg of endotoxin per mouse.

DISCUSSION: We conclude from these studies that the depression of hepatic cytochrome P-450 monooxygenase systems is a general property of interferon inducing agents. This conclusion is based on the probability that a casual relationship must exist between interferon induction and maintenance of the steady state of monooxygenase systems in the liver because the twelve agents used to depress monooxygenase activity were selected solely on the basis of their known ability to induce interferon production. This conclusion also

requires that neither the ability to induce interferon, nor the ability to depress monooxygenase activity are common properties of substances in general. Relatively few substances are known to induce interferon and they are represented largely by the agents listed in Table 1. Of the multitude of xenobiotics which have been tested for their effects on hepatic monooxygenase systems, the very great majority either induce or have no effect on the systems. The few agents that depress these systems usually cause gross morphological changes in the liver; this was not the case with the agents used in this study. While it has been shown by others that endotoxin (14) and poly rI · poly rC (15) depress hepatic monooxygenase systems, we believe this is the first report which suggests that the depression of these systems may be a general property of interferon inducing agents.

Interferon is not as innocuous as was once believed. Interferon preparations inhibited cell division in adult mice (16), inhibited the multiplication of allogeneic spleen and syngeneic bone marrow cells in X-irradiated adult mice (17), and killed newborn mice by inflicting severe liver damage (18). Interferon treatment of uninfected cells has also been reported to inhibit the synthesis of macromolecules (19). The depression of hepatic monooxygenase systems by interferon inducing agents may be another example of a specific effect of interferon on the synthesis of cellular macromolecules.

Our findings may have important clinical implications. It will be important to learn whether viral infections cause a depression of drug metabolizing activity in man. If the effect of interferon inducing agents on drug metabolism is as great in man as it is in laboratory animals, viral infections may be expected to cause drug toxicity in subjects who are using drugs chronically or in patients in which drug therapy is initiated in response to the infection. In view of the potency of endotoxin in our experiments, it might also be expected that rates of drug metabolism may be lowered in certain diseases of bacterial origin. Patients are currently being treated with potent interferon preparations and interferon inducing agents; it will be

important to learn what effect these treatments have on rates of drug metabolism in these patients.

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